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The role of the immune system in brain metastasis

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Abstract

Metastatic brain tumors are the most common brain tumors in adults. With numerous successful advancements in systemic treatment of most common cancer types, brain metastasis is becoming increasingly important in the overall prognosis of cancer patients. Brain metastasis of peripheral tumor is the result of complex interplay of primary tumor, immune system and central nervous system microenvironment. Once formed, brain metastases hide behind the blood brain barrier and become inaccessible to chemotherapies that are otherwise successful in targeting systemic cancer. The approval of immune checkpoint inhibitors for several common cancers such as advanced melanoma and lung cancers brings with it the opportunity and obligation to further understand the mechanisms of immunosuppression by tumors that spread to the brain as well as the interaction between the brain environment and tumor microenvironment. In this review paper we define the central role of the immune system in the development of brain metastases. We performed a comprehensive review of the literature to outline the molecular mechanisms of immunosuppression used by tumors and how the immune system interacts with the central nervous system to facilitate brain metastasis. In particular we discuss the tumor-type-specific mechanisms of metastasis of cancers that preferentially metastasize to the brain as well as the therapies that effectively modulate the immune response, such as immune checkpoint inhibitors and vaccines.

Keywords

brain metastasis; immune system; immunotherapy; metastatic melanoma; metastatic lung cancer

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Introduction

Brain metastases (BM) are the most common type of intracranial neoplasm in adults. It is estimated that in the United States 2.0% of all patients with newly diagnosed cancer have brain metastases at the time of diagnosis, with an annual incidence ranging from 21,000 to 43,000 [1]. Autopsy studies have found that this may be a massive underestimation, and that up to one fourth of all cancer patients develop brain metastases before death, a total of 150,000–200,000 annually [2,3]. Incidence of brain metastases has increased in recent years due to a combination of better diagnostic imaging and successful treatment modalities resulting in longer survival times after diagnosis of primary cancer [4]. According to a study of 15,517 patients in Sweden, which found the incidence of BM to double between 1987 and 2006, the cancers most responsible for this increased incidence are breast in women and lung in both genders [5].

The presence of brain metastases can be a devastating development that significantly worsens the prognosis of patient's primary cancer, and in many cases disqualifies them from various novel and cutting edge clinical trials. Median overall survival in patients with brain metastases left untreated is around 5 weeks [4]. Modern treatment modalities can extend this prognosis to 3–18 months, but multivariate analyses show that patients with brain metastases still carry an overall worse prognosis than their BM-free counterparts [4,6,7]. Not only do metastases to the brain worsen prognosis but they also have a negative impact on quality of life, eliciting CNS symptoms such as ataxia, seizures, visual and speech problems, headaches, and cognitive impairment [8]. To successfully and precisely treat metastatic brain tumors, it is of critical importance to understand the basic molecular mechanisms underlying the formation of brain metastases.

In the course of metastasis, cancer cells must break free from the constraints of their primary tumor, migrate into systemic circulation, survive in circulation, extravasate at their destination, and establish a new niche in a foreign environment; evading detection by the immune system throughout the process. In this review, we describe the role of immune system in metastatic progression, the unique microenvironment of brain metastases, how metastatic mechanisms differ between primary tumor types, and the current state of immunotherapies in the treatment of BMs.

Epidemiology

The most common primary tumor that metastasizes to the brain is that of the lung, specifically small cell lung cancers, with 15.8% of patients having brain metastases [1]. The next most common cancers are non-small cell lung cancers (12.8%), breast cancer (0.4%), renal cancer (1.5%), and melanoma (0.7%) [1]. Interestingly, among patients with metastatic disease, metastatic melanoma patients have the highest rate of brain metastases, reflecting its increased propensity to metastasize to the brain [1]. Prostate and breast cancer brain metastases have the best long-term prognoses, compared to colorectal, melanoma, and lung cancer metastases that have the worst prognosis (Table 1) [1].

Improvements in treatment modalities have led to a gradual increase in the survival for patients with BMs. Whole-brain radiation therapy (WBRT) and corticosteroid treatment developed in the mid-twentieth century increased survival times from 4–6 weeks to 4–6 months [2]. However it is associated with cognitive deficits, which results in significant impairment in the quality of life of long-term survivors. As a result, current practice patterns shifted away from use of WBRT to stereotactic radiosurgery (SRS) for oligo-metastasis, defined as limited number of BMs, reserving WBRT as salvage therapy or for disseminated BMs [9–12]. Advancements in neurosurgery led to the development of surgical resection as a treatment modality for patients with limited number of brain metastases, increasing the median survival times to 10–16 months [2]. The current role for neurosurgery is based on consensus guidelines and is supported by recent retrospective studies demonstrating improved local control and overall survival with the combination of surgery and SRS vs. SRS alone for larger brain metastases [13,14]. Modern research has turned towards immunotherapies, using immune checkpoint inhibitors to modulate the immune microenvironment, and cellular therapies to mount an effective response against the tumors. These therapies have shown, in some cases, to dramatically increase survival times in patients with BMs [15,16]. There is a real potential for these therapies to transform how we treat BMs, but they require a solid understanding of tumor microenvironment and how it may be manipulated.

Immune System and Progression of Metastasis

Stephen Paget first developed the seed and soil theory of metastasis in 1889. He observed a nonrandom pattern of metastases on autopsy in women with breast cancer and hypothesized that this was due to the metastatic cells, what he referred to as the “seeds,” having a greater proclivity for certain organs, the “soil” [17]. A greater understanding of mechanisms of metastasis has made clearer the requirements needed for a cell to free itself from the primary tumor’s constraints, enter the blood stream, survive in the blood stream, invade its destination organ, and then survive in that new environment (Figure 1). One common feature in each of these steps is the need for the metastatic cell to avoid recognition and destruction by the immune system at every step of metastatic progression. Immunosuppressive mechanisms are vital to the survival of the seed, and the immune system is a key driver in creating the fertile soil for these cells to take hold. It is important to understand these mechanisms in the context of brain metastases to design novel and targeted immunotherapies to treat them.

Seed: Primary tumor microenvironment

Tumors can impact the immune response through manipulation of metabolites and stromal components in their microenvironment. One way this is done is through overexpression of IDO, an enzyme that metabolizes tryptophan to kynurenine, effectively depletes tryptophan in the microenvironment [18]. Depletion of tryptophan activates a stress response in T-cells that inhibits their proliferation and induces an immunosuppressive Treg phenotype [19]. Additionally, the release of kynurenine in this process further promotes Treg differentiation, as well as an immunosuppressive phenotype in the professional antigen presenting cells

(APCs), DCs and macrophages, causing them to secrete more regulatory cytokines such as IL-10 and TGF β [19].

Stromal components also inhibit an effective antitumor immune response. The tumor stroma consists of fibroblasts, specialized mesenchymal cells distinct to the tissue environment, vascular endothelial cells, pericytes, and extracellular matrix (ECM) components. These components not only provide a physical barrier to entry by immune cells, they also actively suppress the immune system by impeding antigen presentation to T-cells [20]. Fibroblasts also have a role in tumor progression and metastases, secreting tissue growth factors that can promote tumor growth and matrix metalloproteases (MMPs) that can degrade the ECM and promote growth, invasion, angiogenesis, and metastasis [21]. Lastly, mesenchymal stromal cells can produce TGF- β and soluble decoy T-cell ligands that dampen the immune response and impede T-cell contact with tumor cells [22].

Involvement of immunosuppressive immune cells

Counter regulatory mechanisms, normally used to resolve an inflammatory response are taken advantage of by tumors, allowing immune evasion and promoting their survival. MDSCs, immunosuppressive cells from an immature myeloid lineage, are induced by inflammation and necrosis within the TME and have multiple ways of downregulating the antitumor immune response [23]. These cells deplete amino acids in the TME, starving T-cells and inhibiting signaling pathways required for their activation (Figure 2) [24]. They also produce nitric oxide (NO) through expression of nitric oxide synthases (NOS2 and NOS3), which inhibits IL-2 signaling and T-cell activation [24]. Reactive oxygen species (ROS) are also produced, damaging proteins, lipids, and nucleic acids and enhancing apoptosis of T-cells. Furthermore, the ROS react with NO forming peroxynitrite, which nitrosylates TCRs and MHCs, disrupting their interaction and rendering cancer cells resistant to cytotoxic T-cell responses [24]. MDSCs also express the regulatory cytokine IL-10 and TGF- β , inducing Tregs and M2 polarized, immunosuppressive macrophages [24].

Tumor associated macrophages (TAMs) also contribute to immunosuppression within the TME. In general, macrophages maintain one of two polarization states: M1 and M2. M1 macrophages contribute to a classic Th1 response, producing inflammatory mediators directed against pathogens and tumor cells, while M2 macrophages contribute to immunosuppression and repair. Tumor associated macrophages (TAMs) take on a pro-tumoral M2 phenotype, producing factors involved in growth, extracellular matrix remodeling, angiogenesis, and immunosuppression [25]. Multiple tumor-derived factors drive the recruitment of these macrophages including VEGFA, CCL5, CCL9, CCL18, CCL2, and CSF1 [25–27]. The tumor-promoting role of TAMs is illustrated by the finding that high CSF concentrations in tumors is associated with poor prognosis in breast cancer patients [26]. These TAMs are poor antigen presenting cells, expressing HLA-G and HLA-E, inhibitory MHC-I molecules that inhibit NK cell and T-cell lysis [27]. They also contribute to the immunosuppressive TME through the expression of co-inhibitory molecules PD-L1 and PD-L2, IL-10, TGF β , and chemokines (CCL5, CCL20, and CCL22) that act to recruit Tregs within the TME [27–29]. Additionally, they are highly involved in

the processes of metastasis through extracellular matrix remodeling (TGF- β , MMP-2, MMP-9, LL37, SR-A, Cathepsins) and angiogenesis (VEGF, PDGF) [25,27].

Dendritic cells, the most potent professional APCs, are often divided into 2 main subsets: myeloid dendritic cells (mDCs, CD11c+) and plasmacytoid dendritic cells (pDCs, CD11c-) [30]. The mDCs are present in non-lymphoid tissues, and normally promote a Th1 T-cell response through the expression of IL-12. Conversely, pDCs are present in secondary lymphoid organs, and classically produce IFN- α in response to viral infections [31]. DCs can be further classified as mature or immature, with immature DCs showing decreased co-stimulatory molecule and cytokine expression, leading to a more immunosuppressive phenotype [30]. DCs in the TME tend to be more immature, as the TME inhibits their differentiation [32]. This decreases their ability to present tumor antigens to T-cells and provide the necessary co-stimulation, while actively suppressing the anti-tumor immune response through the expression of IL-10 and TGF β and subsequent promotion of Tregs [32,33]. Additionally, pDCs that are recruited into tumors express a more tolerogenic phenotype, producing IDO and ICOSL, favoring Treg recruitment [31,33]. These pDCs, normally potent IFN- α producers, are poor type I IFN producers in the TME, due to the influence of TGF- β and TNF- α expressed by the tumor cells [31]. They also suppress T-cell proliferation in the TME through the production of granzyme B [31,34]. Notably, pDCs are able to promote anti-tumor immune responses if they are properly stimulated, making them a desirable target for immunotherapies, but the inhibitory nature of the TME induces a pro-tumoral pDC phenotype [31].

The last major cell type involved in creating the immunosuppressive TME is the regulatory T cell. Normally vital to immune homeostasis and induction of tolerance to self-antigens, these cells are potent regulators of the immune system within the TME. They accomplish this immunosuppression through a variety of mechanisms. They release copious amounts of regulatory cytokines (IL-10, IL-35, and TGF β) while expressing numerous co-inhibitory receptors (CTLA-4, PD-1, LAG-3, TIM-3, ICOS, TGIT), and they consume IL-2 in the TME [35]. This prevents the activation of Th1 T-cells and suppresses the antitumor immune response. Furthermore, these cells can be directly cytotoxic to immune cells through the production of perforin and granzyme [35].

Transit (Seed to Soil): Intravascular Microenvironment

Once metastatic tumor cells break free from the primary tumor and enter circulation, they face a new set of challenges in the circulation. Mechanical stress is constantly threatening the circulating tumor cells (CTCs) in the form of sheer forces due to blood flow, and compression when entering small capillaries [36]. Tumors combat this through the formation of cell-platelet and cell-cell microaggregates, making them resistant to mechanical stress [36]. Additionally, through inhibition of the apoptotic signaling pathways these cells not only resist mechanical stress-induced apoptosis, but apoptosis as a result of loss of integrin signaling [36]. Overexpression of the transmembrane protein pannexin-1 inhibits apoptosis induced by mechanical stress [37], and activation of pro-survival pathways like PI3K/Akt counteract the apoptotic effects of integrin signaling loss [38].

In addition to resisting apoptosis induced by a drastic change in environment, the CTCs must avoid immune cell recognition. This is more difficult than in the cells' immunosuppressive primary tumor environment, as the circulation contains numerous peripheral immune cells primed for surveillance. Here platelets also play a role, shielding the CTCs from immune recognition [36]. The role of platelets goes beyond physical shielding of the tumor cells though, they also directly inhibit NK cells through expression of TGF- β , downregulating NKG2D on NK cells [39]. CTCs themselves can avoid recognition by NK cells through cleavage of MICA and MICB on their surface, NKG2D ligands used by NK cells to recognize and kill tumor cells [40]. Lastly, platelets can transfer normal MHC class I molecules to the surface of tumor cells, shielding them from cytotoxic T-cells [41].

In addition to platelets, macrophages play an essential role in protecting CTCs. Platelets and fibrin around CTCs recruit macrophages, which are a required component for the survival of the tumor cells in circulation [42]. One proposed mechanism through which macrophages enhance CTC survival is through expression of $\alpha 4$ -integrin, interacting with VCAM-1 on the CTC surface and providing a survival signal [43]. The macrophage-CTC interaction is not only important in intravascular survival of CTCs, it is involved throughout the entirety of the metastatic cascade. CTCs induce a macrophage phenotype that promotes intravasation of tumor cells from the primary tumor [44]. Furthermore, macrophages facilitate extravasation and colonization of CTCs in distant sites [42,45].

Soil: Development of Metastatic Niche

Once a tumor cell has released itself from the primary tumor, invaded the surrounding stroma, crossed the endothelial barrier into the systemic circulation, and made its way to the vessels in the brain, it arrests in microvasculature at vascular branch points. The physical limitations of the tumor cells within small vessels facilitates the formation of attachment points between the endothelium and the cell [46]. These attachment points are formed using interactions between selectins, integrins, cadherins, CD44, and ICAMs/VCAMs [46,47]. Exactly which receptors and linkages are used is highly complex, and depends on the vascular bed, the type of cancer cell, and interactions with a multitude of cell types within the microenvironment [46].

Once attached, the metastatic cell must migrate through the endothelial cell junction. Breaking down these junctions involves the destruction of junctional adhesion molecules, occludins, and claudins by proteolytic enzymes like seprase and cathepsin S [48,49]. After breaking through the endothelial barrier, the metastatic cells encounter the basement membrane. Breakdown of this barrier is similar to the mechanisms used by the cell to break from the primary tumor [46]. After BBB invasion it is thought that instead of moving into the brain parenchyma, these cells grow along the microvasculature, a propensity known as "vascular cooption" [50]. This is crucial for the establishment of microcolonies. Interactions between integrins on the metastatic cells and the vasculature basement membrane provide the growth and survival cues needed for the new metastatic tumors to grow [50].

Role of immune cells in establishing brain metastasis

It has been questioned whether resident immune cells in the brain contribute to or combat the establishment of BMs. On one hand, around 99% of metastatic cells that enter the brain fail to grow and form macrometastases [51], indicating that there are antagonistic processes inhibiting their establishment. On the other hand, studies have shown that resident immune cells can contribute to a favorable microenvironment, allowing the colonization of new BMs [52–59] (Figure 3). Microglia, both the resident immune cells of the brain and bone-marrow derived monocytes/macrophages in the perivascular spaces, are both facilitators and antagonists to the formation of BMs. Studies have illustrated their ability to combat and lyse tumor cells using NO and shown that upregulating factors like the neurotrophin NT-3, a regulator of microglial cell activation, is associated with increased BM formation [60,61]. This would suggest that microglia are protecting the brain from metastatic cell colonization. However, despite these antitumor mechanisms, microglia have proven to be a driving force in the formation of BMs. Metastatic cells use the cytoplasmic processes of microglia to guide their invasion into the brain [56]. Imaging has revealed the presence of a dense wall of microglial cells at the interface between BMs and the brain parenchyma [52]. Additionally, metastatic cells may modulate the function of microglia. Activated microglia normally produce iNOS and TNF- α , allowing them to lyse target cells [54], but in the presence of BMs microglia show reduced expression of these factors, suggesting the adoption of a polarized M2 macrophage phenotype that facilitates tumor cell proliferation and suppresses cytotoxic functions [54].

Astrocytes play a similar role in the facilitation of BM colonization. Xing et al showed that brain metastases express high levels of IL-1 β under the influence of surrounding astrocytes, which leads to increased Notch signaling in cancer stem cells, promoting their stemness and growth in the metastatic niche [59]. Evidence has also shown the formation of gap junctions between astrocytes and tumor cells that allows for the passage of cGAMP, which activates the STING pathway in astrocytes and promotes expression of IFN α and TNF α to further facilitate tumor growth [53]. The production of ECM-degrading factors like heparanase and matrix metalloproteinases (MMPs) by astrocytes also contributes to invasion of BMs by promoting migration of these cells [55,57]. Overall, these cells that normally protect the brain from foreign invaders end up facilitating the entry and colonization of the brain by metastatic cells.

The primary tumor is also able to influence the immune composition of the premetastatic soil, facilitating the entry and survival of metastatic tumor cells. Liu et al. showed in a mouse model of breast cancer brain metastasis that a primary breast tumor can induce the presence of CD11b⁺Gr1⁺ myeloid cells in the brain, who in turn express the inflammatory chemokines S100A8 and S100A9 that are able to attract tumor cells to the brain [62]. Not only do these myeloid cells induced by the primary tumor have the ability to attract tumor cells to the brain, they can also contribute to their survival. Reports have shown high expression of CCL9 in the same CD11b⁺Gr1⁺ myeloid cells in the premetastatic lungs of mice with breast cancer and melanoma. This CCL9 expression contributed to tumor cell survival and metastasis in a TGF- β -dependent manner [63]. In addition to myeloid cells,

mast cells have also been shown to contribute to the establishment and survival of BMs, through expression of IL-8, IL-10, VEGF, and MMP2 [64].

Tumor Specific Metastasis Mechanisms

Although, there are several steps of the metastatic cascade that are common to all cancer types, there are unique properties of certain types of cancers that renders them more susceptible to form brain metastasis compared to others.

Breast

Of the three subtypes of breast cancer, the HER2-positive and triple-negative subtypes (human epidermal growth factor receptor 2 and estrogen receptor/progesterone receptor/HER2 negative) are the most aggressive and likely to metastasize to the brain. Having a triple-negative cancer portends the worst survival, in part because of the increase tendency for brain metastasis these patients [65,66]. Almost half of the patients with advance triple-negative breast cancer develop brain metastases. Based on the pre-clinical studies this phenomenon has been attributed to triple-negative breast cancer induced disruption of the BBB and migration of tumor cells in the brain parenchyma [67,68]. It has been postulated that during the metastasis process, breast cancer cells (BCC) undergo epithelial-to-mesenchymal transition to enter the blood stream and then reverse the process with a mesenchymal-to-epithelial transition after arriving at brain tissue to form metastases [69]. Evasion of the immune defenses while in the blood stream must also occur for BCCs to make it to the brain vasculature. Racila et al demonstrated that single nucleotide polymorphism variations in C1qA, a subunit of the C1 complex, which recognizes immune complexes and initiates the classical pathway of complement activation, were associated with increased metastasis to the brain, bone, and liver in breast cancer patients. Although this polymorphism has comparable incidence in the normal population without breast cancer, patients that had breast cancer with distant metastases were more likely to have the SNP than those with non-metastatic breast cancer, indicating that the ability to evade opsonization by inhibition of complement activation has a role in spread to distant sites [70].

Once in the vasculature, BCCs upregulates surface cathepsin S expression that facilitates crossing of the BBB through lysis of JAM-B, a key component of tight junctions. This phenomenon has also been observed in primary breast tumors that have spread to the brain, not solely in BMs [49]. Another protein that is upregulated in triple negative breast cancer as well as lung adenocarcinoma and is linked to poor prognosis is mesothelin. A positive correlation was observed between detectable IFN-gamma in response to mesothelin and increased survival in whole blood analyses of patients with epithelial cancers (lung, breast, ovarian, melanoma), suggesting that mesothelin is an important tumor-associated antigen in brain metastases [71].

BCCs can manipulate microglial activation through expression of neuronal proteins. When expressed on the surface of BCCs, NT-3 promotes the formation of macroscopic lesions in the brain. It appears to play a key role in the switch back to an epithelial phenotype, allowing the BCCs to survive in the brain parenchyma after having invaded the abluminal basement membrane. Additionally, NT-3 encourages the proliferation of BCCs in the brain as shown

in mouse models with injected human tumor cell lines [61]. Immunomodulation by NT 3 occurs via inhibition of microglia activation. Low levels of microglial activation promote growth of metastatic tumors whereas high levels of activation result in cytotoxicity [54,61,72]. CXCR4 signaling, which also activates microglia, has been found to facilitate invasion of BCC in a mouse model [73]. Differential activation of microglia can induce different helper T cell responses. Investigations of Th2-mediated immune responses within breast cancer have revealed its role in permitting BM growth. Thymic stromal lymphopoietin (TSLP) is a cytokine that mainly promotes a Th2-mediated immune response; depletion of this cytokine resulted in smaller primary tumors yet larger BM burden [74], suggesting that inhibiting the Th2 response may prove useful in treating BMs from breast cancer.

Melanoma

Patients with regionally advanced melanoma, including regional lymph node involvement and those with unknown primaries, are at a significantly increased risk for CNS involvement; as high as 15% of these patients will have BMs [75]. CCR4 (chemokine receptor 4) is expressed on melanoma cells and has increased expression in metastatic melanomas as compared to primary melanoma. Additionally, ligands for CCR4 (CCL17 and CCL22) are expressed by astrocytes, microglia, and brain endothelial cells under stress conditions and when exposed to melanoma-derived supernatants. In their mouse model, Klein et al also found that CCL17 was significantly upregulated 10 weeks post-inoculation of melanoma cells but before any intracranial micro-metastases were detectable, indicating that cutaneous melanoma has a long-distance mechanism which induces upregulation of CCL17, which acts as a chemoattractant to melanoma expressing CCR4+ [76]. CCR4 is also expressed by Th2 cells and Tregs [77] and acts as a chemoattractant for the T-cell populations. This induces tolerance via astrocytic, microglial, and brain endothelial expression of CCL17, and could be another mechanism through which metastatic melanoma evades the immune system. Another possible contributing mechanism of melanoma metastasis was found by Kaur et al. They saw that aging fibroblasts secreted increased amounts of sFRP2, a Wnt antagonist, which ultimately led to an attenuated response to DNA damage in melanoma cells, making them more resistant to vemurafenib, and drove their metastasis [78].

Axitinib, an inhibitor of VEGFR-1, 2, & 3, which induces a response in roughly 20% of those patients treated, was found to increase the number of tumor-infiltrating immune cells (CD45+) in an intracranial melanoma mouse model. Specifically, the sub-population of monocytic myeloid derived suppressor cells (moMDSCs – CD11b+Ly6ChighLy6G-) was increased in the intracranial tumors. These cells were found to have a reduced capacity for immune suppression in both intracranial and extracranial tumors but had acquired an antigen-presenting phenotype only in the subcutaneous tumors [79]. Another study found that once metastatic melanoma reaches the CNS it become significantly more tolerogenic than equivalent tumors in the periphery, an effect mediated by an increase in circulating TGF- β [80].

Lung

Brain metastases from lung cancer are associated with dense accumulations of activated microglia that demarcate the boundary between tumor and adjacent brain tissue. *In vitro* studies, show that dual functions of microglia exist – protective versus cytotoxic responses. When different concentrations of LPS-activated (lipopolysaccharide) microglia supernatant were applied to metastatic lung cancer cells, the cancer cells behaved differently. At lower concentrations, the cancer cells had increased viability; at higher concentrations, viability decreased [54]. In this model both the activation and inactivation of microglia has been observed; increases in numbers as well as transformation to amoeboid/activated shape seen suggest activation while a lack of inducible nitric oxide synthase (iNOS) or TNF-alpha (tumor necrosis factor alpha) expression suggest that inactivation occurs simultaneously within tumor microenvironment [54]. Other studies confirm the presence of increased amounts of peritumoral microglia accumulation in NSCLC brain metastases as compared to melanoma brain metastases, however there is relatively little expression of iNOS and other enzymes involved in free radical production, suggesting that many of these microglia are either inactive or are supportive of the tumor [81]. Sparse T-cell and B-cell infiltrates found within brain metastases suggest that these cells are secondarily recruited and are not necessarily antigen-specific [81]. McGranahan et al found that metastatic squamous cell lung cancers had decreased expression of many HLA class I genes as well as decreased expression of components of the MHC class I molecule, suggesting that the metastatic tumors directly evade the immune system by decreasing the chances of successful T-cell activation [82]. Additionally, recent studies have shown that NSCLC BMs, despite have a higher mutational burden, contain fewer T-cell clones than their primary tumor counterparts, and the majority of the T-cell clones that were found in the BMs differed from those in the primary tumor [83]. This suggests not only a change in tumor immunogenicity following metastasis, but also highlights the ability of the blood brain barrier to inhibit immune responses in the CNS. Mast cells (MCs) have also been found in human BMs (via tryptase staining) with lung, renal, and breast origins. MCs support BM propagation via secretion of immune suppressive cytokines IL-8, IL-10, as well as VEGF and MMP2 [84] that modulate the microenvironment and contribute to metastatic potential in lung cancer patients.

Colon

Metastasis to the brain is a rare complication of CRC, and thus research on the treatment and mechanisms of this metastasis is sparse. Most metastatic colorectal cancer patients develop brain metastases as a late step in the course of the disease and it is associated with poor overall prognosis [85]. With significant improvements in the management of colorectal cancer, the incidence of metastases at previously uncommon sites is suspected to rise [86]. C-X-C chemokine receptor type 4 (CXCR4) and the placental enzyme indoleamine 2, 3-dioxygenase (IDO) have both were found to be upregulated in some colorectal carcinoma (CRC) brain metastases. CXCR4 along with its ligand CXCL12 is involved in lymphocyte trafficking via chemotaxis and its upregulation in CRC has been associated with worse survival. IDO is important in suppressing the maternal T-cell response against the fetus and when observed in primary CRC predicts the formation of distant metastases [87], but otherwise the mechanism by which these two proteins confer worsened survival is unknown. Recent investigations into BM from CRC have otherwise neglected the immune system's

role and have instead focused on the robust connection between the oncogenes in RAS family [88–90].

Immunotherapy for Brain Metastasis

Over past several years immunotherapy has emerged as an effective and robust strategy in the treatment of primary cancers such as lung, melanoma, renal and others [91–94]. Given the prominent role played by the immune system at every step of brain metastasis formation, there has been great interest in targeting the immune system to treat brain metastasis. Many patients with metastatic melanoma and metastatic lung cancers, were reported to have better survival after treatment with immunotherapies; these successes with nivolumab, ipilimumab, and pembrolizumab have improved the outlook for patients with certain metastatic cancers [94–97]. Until recently the clinical trials involving these medications excluded patients with brain metastasis, thus their effects on metastatic brain lesions is largely unknown. The immunotherapy strategies investigated to date include targeting the receptors on T-cells which are required for their activation and inhibition with monoclonal blocking antibodies [95,96], blocking the corresponding ligands on tumor cells and antigen-presenting cells [98,99], administering immune system-activating cytokines such as IL-2 [100], and tumor vaccines [101–104]. Table 2 outlines the current clinical trials that are evaluating immunotherapies for the treatment of brain metastasis.

Immune checkpoint inhibitors

Blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4) in metastatic melanoma has shown great promise in extracranial melanoma metastases by extending survival [94] and is in clinical trials for patients with BM. Expressed on Tregs, CTLA-4 binds to CD80 and CD86. It is in direct competition with CD28 (a T-cell co-stimulatory molecule) and induces T-cell anergy or suppression, thereby producing immune tolerance. In the tumor environment this can be critical for survival. Synder et al created a bioinformatic tool to evaluate binding of exomic mutations with MHC class I molecules in patients with metastatic melanoma treated with ipilimumab and generated a 101-tetrapeptide signature that confers benefit from CTLA-4 blockade [105]. These neoepitopes did not result from a high mutational load as the amount of mutations did not correlate with benefit from CTLA-4 blockade. Many of these epitopes were homologous to bacterial and viral antigens already present in the Immune Epitope Database, suggesting that the patients who will benefit best from anti-CTLA-4 therapy are those whose tumors express epitopes similar to antigens already recognized by T-cells [105]. Interestingly in contrast to melanoma BM, there is no CTLA-4 expression on TIL in breast cancer BM; however, breast BMs commonly express PD-L1 and PD-L2 [93]. As breast cancer has not been typically thought of as an immunogenic cancer, these findings suggest that there is an untapped well of potential for therapy with immune checkpoint inhibitors.

PD-ligands transmit an inhibitory signal when binding to PD-1 receptors on T-cells, leading to reduced proliferation and apoptosis. The PD-1 antibodies pembrolizumab and nivolumab have been approved for advanced melanoma and nivolumab has been additionally approved for metastatic NSCLC [95–97]. Studies of metastatic tumor expression of PD-L1

have found that melanoma BM express lower levels of PD-L1 as compared to the primary tumor and extracranial metastases. The brain also has lower CD8+ T-cell content than other areas of metastases. Higher PD-L1 expression and T-cell content is associated with improved survival in melanoma [98]. In addition to variability in PD-L1 expression and TIL density seen in extracranial versus intracranial tumors, levels of PD-L1 expression differ between types of melanoma; with only 10% of uveal melanomas expressing PD-L1, while expression is seen 62% of chronic sun damaged melanomas [106]. This may account for the variability in response rates of melanoma to checkpoint inhibitors. Others have demonstrated that renal carcinoma BMs also have high amounts of CD8+ lymphocytes, similar to melanomas but Foxp3+ cells (Tregs) are found in rather low quantities in all tumors [99]. These findings suggest that expansion of the indications for immune checkpoint inhibitor therapy may be warranted; further investigation will need to be done to determine the viability of these therapies in other cancers with BMs. Furthermore, biomarkers of response to these checkpoint inhibitors are needed to predict which patients will benefit from these therapies. Some suggested markers have been the tumor's mutational load or the burden of copy number loss, which have been shown to be correlated with clinical outcomes in patients treated with PD-L1 and CTLA-4 inhibitors [107,108].

Another factor in the use of checkpoint inhibitors is their interaction with the human gut microbiome. Recent studies have shown significant differences in the microbiome of those patients who responded to therapy and those who did not. The responders had higher alpha diversity, a significantly larger population of Ruminococcaceae, and an enrichment of anabolic pathways, all leading to more favorable antitumor immunity [109]. Additionally, this phenotype could be reconstituted in germ free mice through a fecal transplant. Another study found that the microbiomes of metastatic melanoma patients who responded to anti-PD-L1 therapy were richer in *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* compared to non-responders; and again the improved anti-tumor immunity and greater efficacy of anti-PD-L1 agents seen in these patients could also be seen in germ free mice who received a fecal transplant from them [110]. These studies suggest that it is possible to enhance a patient's response to immune checkpoint inhibitors through modification of their microbiome.

Tumor vaccines for brain metastasis

Most vaccines are used prophylactically to induce an adaptive immune response against a specific antigen, usually antigens present on pathogens one wants to protect against. However, in the case of using vaccines against tumors the goal is to induce a therapeutic adaptive immune response, as opposed to a prophylactic one. Multiple vaccine types have been proposed as candidates to treat intracranial tumors: peptide vaccines, adoptive T-cell transfer, and DCs primed with tumor antigens are the major examples. These strategies have been extensively investigated for the treatment of high grade gliomas and glioblastomas, yet not so for brain metastases [111]. In the context of glioblastoma, a large phase III clinical trial showed that addition of an autologous tumor lysate-pulsed dendritic cell vaccine to be safe and may increase overall survival [112]. In the context of other cancers, the exclusion criteria for patients enrolled in trials employing vaccination strategies for other malignancies usually preclude the enrollment of patients with CNS involvement [113]. Though the

existence of large trials employing vaccines against BMs is lacking, there have been a few select case reports and small clinical studies performed.

Peptide vaccines induce an antitumor immune response by being taken up by APCs and being presented to T-cells, which then travel to the brain to recognize and kill the tumor cells based on the antigen presented. One group recently used peptides from IDO and survivin to vaccinate melanoma patients in a phase II study, in combination with TMZ. The study was done in 17 patients, 8 of which had brain metastasis, and their results showed a clinical benefit in 18% of the patients, assessed by PET-CT, with one patient showing tumor regression [114]. While the clinical benefit of this study was underwhelming, the peptide vaccine was able to induce vaccine-specific immune responses. Peptide vaccines still face multiple challenges that researchers are attempting to overcome. Unfortunately, tumor heterogeneity and antigen selection lead to the proliferation of tumor cells without the antigen being vaccinated against. This mechanism of resistance is being addressed by next-generation multi-peptide vaccines [115]. An additional challenge is inducing a sufficient immune response to generate the necessary number of activated APCs and subsequent anti-tumor T-cells [115].

DC vaccines have also been used with some success. They are formed by isolating a patient's own DCs, electroporating them with mRNA of the antigen(s) of interest, expanding and activating these cells, and then injecting them back into the patient, where they can travel to lymph nodes and activate antitumor T-cells. These vaccines have the advantage of activating the APCs *ex vivo*, leading to more sufficient activation than seen in peptide vaccines. Additionally, unlike peptide vaccines, DC vaccines have shown the ability to evolve alongside the tumor and increase the diversity of antigens presented over time [116]. In one study of 12 patients with metastatic renal cell carcinoma, one patient with four brain metastases demonstrated a complete and durable regression after being treated with a DC vaccine [117]. Success was also seen in a phase I/II trial where 20 patients with metastatic melanoma, 3 with BM, were treated with DCs incubated with irradiated melanoma cells and GM-CSF [118]. They achieved an overall survival rate of 95% at a median follow-up of 13.8 months and reported one case in which a woman with a BM remained in remission 36 months after starting the therapy. In a follow up randomized phase II trial by the same group, 42 patients with melanoma were randomized to DC vaccine vs. tumor cell vaccine and followed for 5 years. Their results showed that patients treated with DC vaccine had 70% reduction in the risk of death and double the median survival [119]. The difficulties in using DC vaccines lies in the localization of DCs to lymph nodes; only a small fraction make it to a lymph node to present the antigen and there are barriers to entry and presentation from the CNS lymphatics and BBB [115]. However because of biological heterogeneity and neo-antigens resulting from each patient's cancer mutational landscape, autologous tumor derived tumor associated antigen loading of DCs has the potential to induce a more diverse and effective anti-tumor immune response. It is clear that DC vaccines are able to exert an anti-tumor effect in the CNS in people with BMs, but larger trials looking exclusively at patients with BMs are needed.

Combined Stereotactic Radiosurgery and Immunotherapies for Brain Metastases

One question that arises in the implementation of immunotherapies to treat BMs is how they interact with the current standard of care; in the case of brain metastases this is stereotactic radiosurgery (SRS). Systemic radiation is thought to be antagonistic to immune therapies, as it inhibits the proliferation of peripheral immune cells and induces lymphopenia [120]. However, with the advent of SRS, a more concentrated, focal dose of radiation can be delivered to a tumor bed and can not only have an antitumor effect at that site but also at distant metastatic sites not targeted by radiation; a phenomenon known as the abscopal effect [121]. It is thought that this effect is primarily immune mediated [122], as radiation is able to increase antigen availability and visibility as well as activate important immunostimulatory pathways like cGAS-STING [123]. The immune modulatory effects of SRS suggest that there is an opportunity for synergism between these two modalities.

In 2012 Knisely *et al.* published a retrospective analysis of 77 patients with melanoma BMs who had received SRS and showed significant improvements in the median survival of patients who had also received the CTLA-4 inhibitor Ipilimumab, from 4.9 months to 21.3 months [15]. The benefits of combined SRS and immunotherapies for melanoma BMs has been subsequently confirmed in multiple studies [124–126], and some have even shown their treatment with SRS and immune checkpoint inhibitors to be superior to SRS and chemotherapy or BRAF/MEK inhibitors [127,128]. In addition to melanoma, concurrent SRS and systemic immunotherapy has also shown a benefit in the treatment of NSCLC BMs [129,130]. Studies are needed to investigate if this benefit may also be conferred in those patients with BMs from breast, renal, and colon cancers.

Future of Immunotherapy in Brain Metastasis

With the recent success of the combined ipilimumab and nivolumab trials, it appears checkpoint inhibitors will remain at the forefront of immunotherapy in the treatment of BMs. Besides the massive success these antibodies have shown, they are also technically and logistically more feasible in the treatment of BMs. Premade antibodies that can be stored *en masse* have a distinct benefit over culturing and expanding DC or T-cell populations, or even in producing patient-specific peptides for vaccination. Even though with limitations, tumor vaccines can be optimized to increase their efficacies. Development of an adaptive immune response is a slow process, which makes it difficult to use when trying to outpace the growth of the tumor. This does not mean that these approaches are ineffective though. Multiple studies have demonstrated their efficacy and feasibility and it is likely that in the future the active immunization could be used in concert with the passive antibody treatments.

Using these therapies in combination provides a distinct benefit and likely reduces the probability of escape variants. In the future, immunotherapies for brain metastases will likely be used like treatments for HIV: using 4 or 5 different drugs at once that all target discrete mechanisms, making it harder for the resistant variants to emerge. The challenge will be mitigating the effects of immune overactivation in the CNS, which is known to have devastating side effects itself. There is also the advent of exciting new technologies on the

horizon. One exciting development is the advent of so called “Y-traps,” checkpoint inhibitors fused to a TGF β receptor domain. This allows for a single molecule to simultaneously block checkpoint inhibition, while removing the inhibitor molecule TGF β from the TME. This combinatorial effect, combined in a single molecule, allowed for greater inhibition of Tregs and greater antitumor response than seen with PD–L1 inhibitors alone [131]. It will be interesting to see in the future if this bifunctional antibody approach can be used on other immune regulators or expanded to target an even greater number of molecules at once.

Besides the development of new therapies, there is also currently an attempt to improve the existing therapies. One way this is being done is through the development of biomarkers that will accurately predict which patients will respond to specific immunotherapies. Exploring new immune markers like PD–L1 with immunohistochemistry, the assessment of mutation load and microsatellite instability, the characterization of TILs, and the gene expression profiles of tumors all serve as ways to parse out who will best respond to which immunotherapies [132].

Conclusion

Cancers that metastasize to the brain have mechanisms to avoid immune detection and subvert the immune system for their own benefit. They alter T–cell ligand and co-stimulatory molecule expression, activate and suppress microglia, activate macrophages, secrete anti-inflammatory cytokines, downregulate proteins needed for antigen presentation, and upregulate angiogenic factor expression. The brain microenvironment provides an interesting context to the above changes: astrocytes prevent microglial activation from getting out of control in the setting of injury as well as during metastasis growth and yet some microglia have been implicated in promoting tumor growth as opposed to being too weak to impede it. Specific cancers have been found to have some differing mechanisms of metastasis to the brain and some that are shared between a few individual types. So far there has been no one underlying mechanism that explains all metastasis to the brain. Macroscopically, multiple different mechanisms combine in each type of primary cancer to produce the same result; further studies will delineate the differences and commonalities between mechanisms of various cancer types. Better and more precise understating of the metastatic process and host immune system will open the door for development of successful future immunotherapeutic options for BMs.

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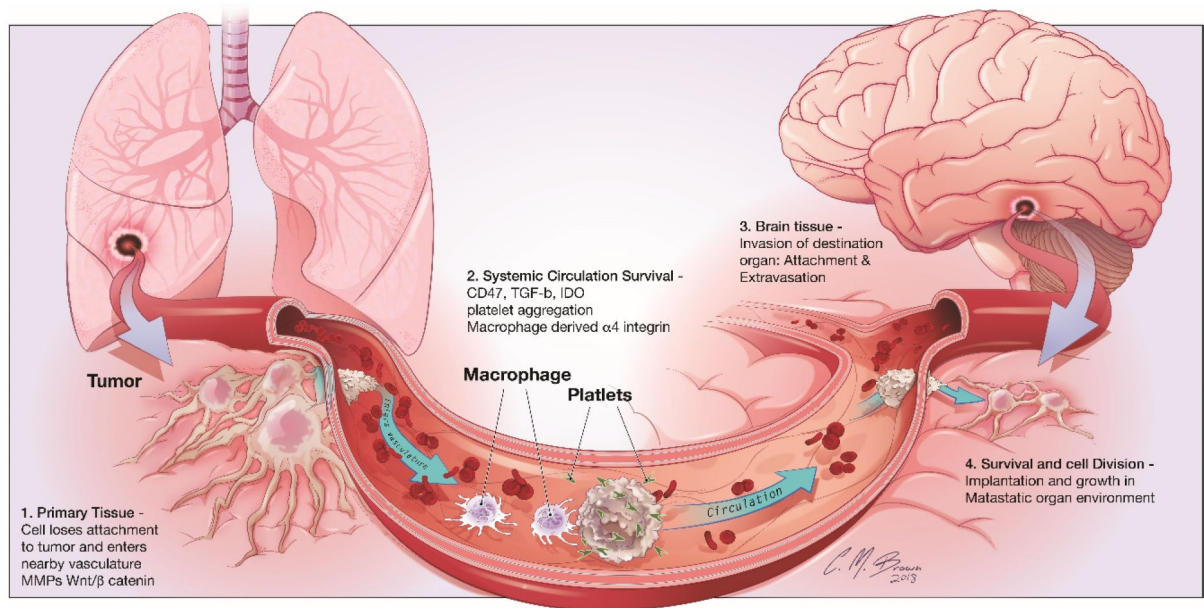


Figure 1. Steps Involved in Brain Metastasis.

Brain metastasis cascade involves four major steps: 1) Detachment of the metastatic cell from the primary cancer, 2) Survival in systemic circulation, 3) Invasion in the brain parenchyma and 4) survival in the CNS microenvironment.

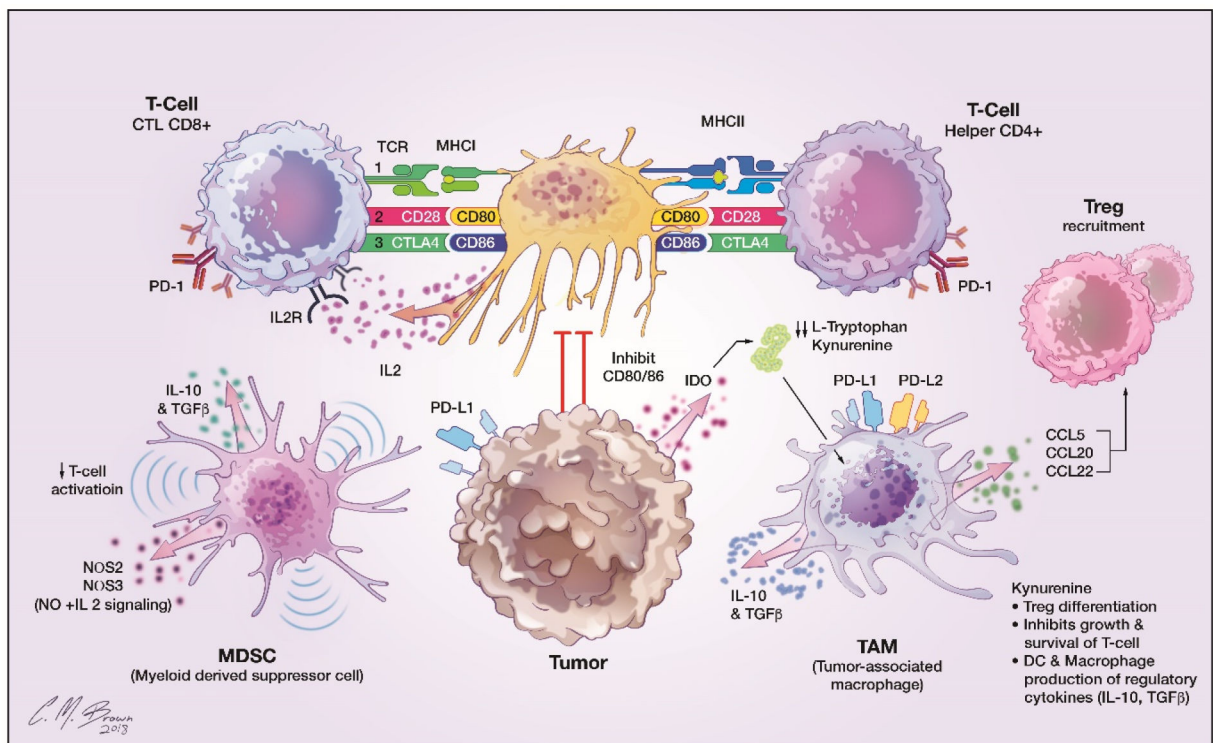


Figure 2. Immune Cell and Tumor Cell Interaction.

Tumor cells can actively manipulate the tumor immune microenvironment by interacting with dendritic cells (DCs), tumor associated macrophages (TAMs), myeloid derived suppressor cells (MDSCs) and T cells. Overall goal of tumor mediated immune cell manipulation is to achieve immunosuppressive microenvironment.

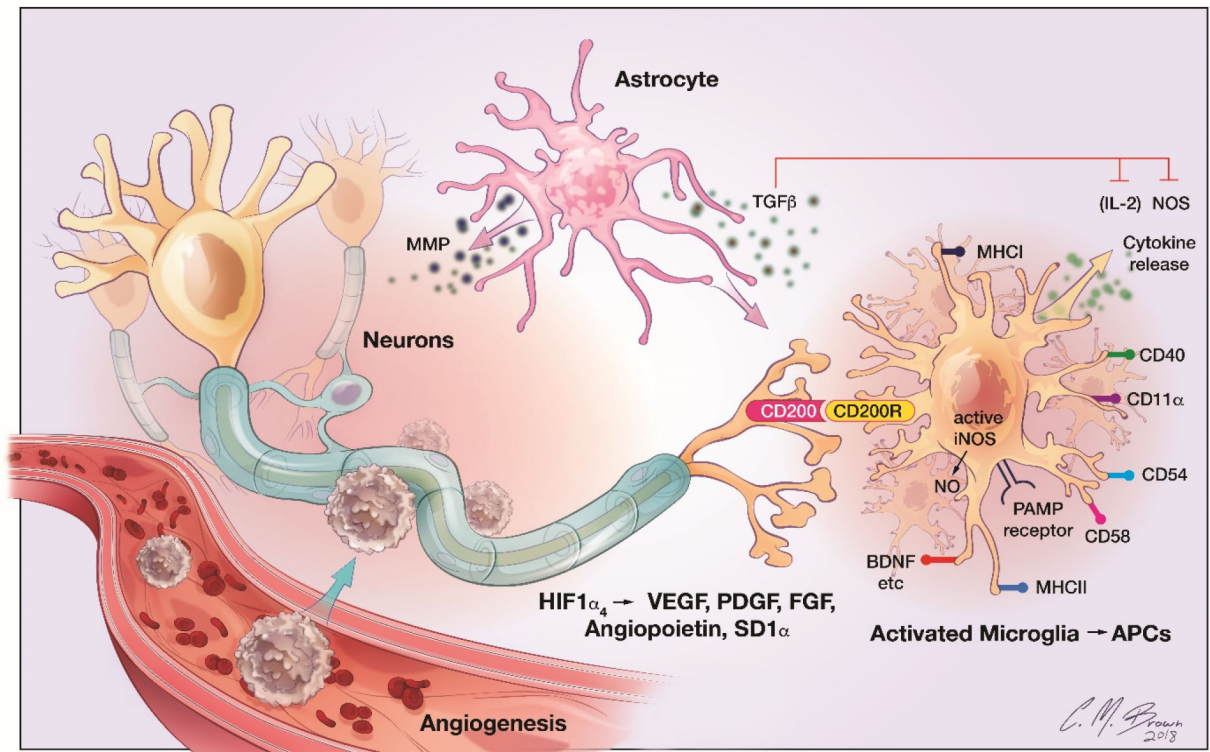


Figure 3. CNS Microenvironment of BM.

Upon invasion of the brain parenchyma tumor cells predominantly interacts with activated microglia and astrocytes to establish a new metastatic niche.

Table 1.

Proportion of patients with brain metastasis at time of initial diagnosis, median survival after brain metastasis diagnosis, incidence of leptomeningeal disease, and metastatic pattern in patients with SCLC, NSCLC, adenocarcinoma of the lung, breast cancer, renal cancer, colorectal cancer, and melanoma [1,133–137].

Type	Incidence of Brain Metastases at Initial Diagnosis (%)	Incidence of Brain Metastases Among Patients with Metastatic Disease (%)	Median Survival after Brain Metastasis Diagnosis (months)	Incidence of Leptomeningeal Disease (%)
Small Cell Lung Cancer	15.83	22.43	6.0	10–25
Non Small Cell Lung Cancer NOS	12.81	22.56	4.0	1–3.8
Breast Cancer	0.41	7.58	10	5
Renal Cancer	1.48	10.84	5	0.03
Colorectal Cancer	0.27	1.36	6	0.058
Melanoma	0.65	28.16	6	22–46

Table 2.

Clinical trials investigating immunotherapies in brain metastasis.

Trial Name	Interventions	Status	Location
Improved Therapy Response Assessment in Metastatic Brain Tumors	Diagnostic Test: Magnetic Resonance Imaging <ul style="list-style-type: none"> Radiation: Stereotactic Radiosurgery Drug: Ipilimumab, nivolumab or pembrolizumab 	Recruiting	<ul style="list-style-type: none"> Oslo University Hospital, Oslo, Norway
Proteome-based Immunotherapy of Lung Cancer Brain Metastases	<ul style="list-style-type: none"> Biological: Dendritic vaccine, allogeneic hematopoietic stem cells, cytotoxic lymphocytes Biological: Dendritic vaccine, autologous hematopoietic stem cells, cytotoxic lymphocytes 	Enrolling by Invitation	<ul style="list-style-type: none"> ZAO "NeuroVita Clinic of Interventional and Restorative Neurology and Therapy", Moscow, Russian Federation
Proteome-based Immunotherapy of Brain Metastases From Breast Cancer	<ul style="list-style-type: none"> Biological: Dendritic vaccine, allogeneic hematopoietic stem cells, cytotoxic lymphocytes Biological: Dendritic vaccine, autologous hematopoietic stem cells, cytotoxic lymphocytes 	Enrolling by Invitation	<ul style="list-style-type: none"> ZAO "NeuroVita Clinic of Interventional and Restorative Neurology and Therapy", Moscow, Russian Federation
Anti-PD 1 Brain Collaboration + Radiotherapy: The ABC-X Study	<ul style="list-style-type: none"> Drug: Ipilimumab Drug: Nivolumab Radiation: Stereotactic Radiotherapy Radiation: Whole Brain Radiotherapy 	Not yet recruitin	<ul style="list-style-type: none"> Westmead Hospital, Sydney, New South Wales, Australia Melanoma Institute Australia, Wollstonecraft, New South Wales, Australia Princess Alexandra Hospital, Woolloongabba, Queensland, Australia Royal Adelaide Hospital, Adelaide, South Australia, Australia Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia
A Study of Fotemustine (FTM) Vs FTM and Ipilimumab (IPI) or IPI and Nivolumab in Melanoma Brain Metastasis	<ul style="list-style-type: none"> Drug: Fotemustine Drug: Fotemustine and Ipilimumab Drug: Ipilimumab and nivolumab 	Recruiting	<ul style="list-style-type: none"> Medical Oncology, Cancer Institute "Giovanni Paolo II", Bari, Italy Medical Oncology, Pope Giovanni XXIII Hospital, Bergamo, Italy National Institute for Cancer Research, Genoa, Italy Immunotherapy and Somatic Cell Therapy Unit, Scientific

Trial Name	Interventions	Status	Location
			<p>Institute of Romagna, Meldola, Italy</p> <ul style="list-style-type: none"> Surgical Oncology, National Cancer Institute, Milan, Italy European Institute of Oncology, Milan, Italy Medical Oncology and Innovative Therapy, National Cancer Institute, Naples, Italy Esophageal and melanoma oncology, Istituto Oncologico Veneto, Padua, Italy Medical Oncology, National Cancer Institute "Regina Elena", Rome, Italy Medical Oncology and Immunotherapy Unit, University Hospital of Siena, Siena, Italy S C Dermatology, A.O.U. City of Health and Science of Turin, Turin, Italy
Radiation Therapy with Combination Immunotherapy for Relapsed/Refractory Metastatic Melanoma	<ul style="list-style-type: none"> Drug: Interleukin-2 & Ipilimumab (P-Ib) Drug: Interleukin-2 & Ipilimumab (P-II) 	Recruiting	<ul style="list-style-type: none"> Masonic Cancer Center - University of Minnesota, Minneapolis, Minnesota, United States
Personalized Cellular Vaccine for Brain Metastases (PERCELLVAC3)	<ul style="list-style-type: none"> Biological: Personalized cellular vaccine 	Recruiting	<ul style="list-style-type: none"> Guangdong 999 Brain Hospital, Guangzhou, Guangdong, China
Anti-PD 1 Brain Collaboration for Patients with Melanoma Brain Metastases	<ul style="list-style-type: none"> Drug: Nivolumab Drug: Ipilimumab 	Active, not recruiting	<ul style="list-style-type: none"> Melanoma Institute Australia, North Sydney, New South Wales, Australia Princess Alexandra Hospital, Woolloongabba, Queensland, Australia Royal Adelaide Hospital, Adelaide, South Australia, Australia Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia